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Art. V.—*Botrytis Corm Rot of the Gladiolus—its Cause and Control.*

By G. C. WADE, B.Agr.Sc.

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Abstract.

A serious corm rot of the Gladiolus, caused by a species of *Botrytis*, probably identical with *Botrytis gladioli* Kleb., is described. The same fungus infects the leaves and flowers of the Gladiolus. It overwinters as sclerotes which germinate, producing conidia, under certain conditions. Infected flowers develop abundant conidia. Corms are infected after digging, the fungus entering through the cut stem end or the old corm. It spreads along the vascular bundles and finally causes extensive rotting. The disease may be controlled by dipping with "Hortosan DP," "Zetan," Corrosive sublimate or "Aretan" as soon after digging as possible. The organism has a low optimum and maximum temperature, and the disease may be avoided by digging early. A number of popular varieties are resistant.

Conidia are produced in artificial culture on certain media, under the stimulus of light. The fungus responds to increasing carbohydrate content in the presence of vitamins or plant extracts, but not in their absence. Starch accumulates in actively growing lesions, but in arrested lesions the accumulated starch disappears and a suberinised layer develops between the healthy and diseased tissue. The phloem tissue of infected vascular bundles is destroyed before the xylem tissue. Infected corms develop an indicator pigment by reaction between the living corm tissue and the fungus.

Introduction.

In June, 1940, specimens of Gladiolus corms, exhibiting a soft rot condition, were forwarded to the Department of Agriculture by a grower at Kalorama, Victoria. The condition did not resemble the Gladiolus diseases common in Victoria up till that time, and investigations were commenced to determine its cause and control. As will be demonstrated, in this article, the disease was found to be *Botrytis* Corm Rot.

This disease was first described from Canada in 1927 (5) and has been mentioned in Canadian literature several times since that date (14, 26). Drayton stated that it was common in Holland in 1929 (12), and according to van Poeteren (54) it is gaining ground in that country. Moore (37) first noted the disease in England in 1927. He observed foliage symptoms in Holland in the following year. It was recorded from Long Island, New York, in 1941 (11).

In 1934, Noble *et al* (40) recorded a leaf and stem blight caused by a species of *Botrytis* in New South Wales but, as no mention was made of corm symptoms, this appears to be distinct from the disease described here.

Dimock (10) described an epiphytotic of a leaf and flower blight in Florida which he attributed to a species of *Botrytis*. Tisdale, W. B. (53), has subsequently cast doubt on Dimock's diagnosis and considers the blight was not caused by any organism but by physiologic causes. The lack of corm symptoms and the doubt of the actual cause made it appear a different disease to that described here.

From information obtained from growers the disease was probably present in Victoria for at least a year before specimens were submitted to the Department. It was apparently introduced in imported corms somewhere about 1938-1939. Since the first record from Kalorama it has been recorded from other parts of the Dandenong Ranges, the Geelong district, the Ballarat district, the Mornington Peninsula, and the Metropolitan area of Melbourne. The disease is also present in the coastal regions of New South Wales (correspondence with Drs. C. J. Magee and Lilian Fraser of the New South Wales Department of Agriculture).

Losses caused by the disease have been considerable. Some growers in the Kalorama district have lost over 50 per cent. of the corms of susceptible varieties in years which have been favorable for the occurrence of the disease.

Symptoms of the Disease.

All parts of the plant are affected by the fungus, but from an economic point of view the attack on the corm is the most serious aspect of the disease.

Corm Symptoms.

Corms may exhibit several types of symptoms but, in the opinion of the writer, these symptoms are successive stages in the attack of the fungus on the corm. This view has also been suggested by Moore (37).

In the earliest stage of the disease only the core of the corm is attacked. At this stage there are no obvious external symptoms and affected corms can readily be overlooked in an inspection of corms. A close examination, however, shows a brown discolouration of the basal plate. When the corm is cut the core region shows various stages of a brown rot condition (Plate V., figs. 4-7).

In later stages of the disease the rot travels along the water conducting vessels (Plate V., figs. 5-7) and it is possible for the corm to be extensively rotted internally without obvious external symptoms.

When the disease reaches the surface of the corm, it spreads rapidly producing a soft brown rot. Finally the whole corm may be reduced to a soft, rotten condition. Even at this stage of the disease, the symptoms may not be obvious till the bulb scales are removed. However, an infected corm will feel very soft when squeezed. If the corm is held under humid conditions the fungus produces abundant white, cottony mycelium on the surface of the corm. This mycelium develops large, black sclerotial masses (Plate V., fig. 3). Usually the sclerotes coalesce forming large coralloid bodies. This stage is similar to that illustrated by Hicks (26). The mycelial growth and sclerote development usually occur on the surface of the bulb scales and between the bulb scales and the corm itself.

Under other conditions, which are not completely understood but probably include exposure to light, and to less humid conditions than those which produce the cottony mycelial stage, abundant conidia of *Botrytis* are produced on the surface of the corm. However, this stage is less frequently observed in Victoria than the cottony mycelium-sclerotial stage.

After extended storage the soft, rotten corms gradually dry out to a mummified condition, which usually bears abundant sclerotes on the surface (Plate V., fig. 2).

The extensive internal disorganization of the corm, which in practically all cases involves the entire core region, clearly differentiates this disease from other common Gladiolus diseases such as *Sclerotinia* corm rot, *Septoria* corm rot, and *Penicillium* rot. *Sclerotinia* and *Septoria* produce very hard dry rots, which do not usually penetrate very deeply into the corm. *Penicillium* may produce an extensive soft rot, but it can usually be traced to an injury on the surface of the corm and its commencement cannot be traced to the core region. Thus a positive diagnosis of *Botrytis* corm rot can usually be made from a section of an infected corm.

The symptoms of *Fusarium* Yellows (McCullock, 36) resembles the *Botrytis* disease in several respects. In both diseases the core of the corm is attacked and the disease follows the vascular bundles. This disease has not been recorded in Australia and the author has not had the opportunity of examining specimens, but the presence of sclerotes on *Botrytis* infected corms would distinguish the two diseases. The description in McCullock's paper suggests that *Botrytis* also causes a softer type of rot than *Fusarium*.

Leaf Symptoms.

The same fungus is capable of attacking the leaves of the plants. Affected leaves usually show a large number of small brown spots, which frequently have a reddish margin (Plate VI.,

fig. 13). The spots may coalesce causing larger brown areas and the leaves may die prematurely. Conidia are produced on infected leaves under field conditions, but much less abundantly than on infected flowers. There is no evidence that this phase of the disease is of great importance under Victorian conditions, as it rarely appears until after flowering, when the plants are already senescent. Until they are senescent Gladiolus leaves grow vertically and have a waxy surface. This habit of growth would not favour infection from spores.

Flower Symptoms.

Flowers frequently become infected in the field during periods of humid weather. The fungus causes water-soaked areas on the petals (Plate VI., fig. 16). These areas increase in size rapidly and finally cause the flower to collapse (Plate VI., fig. 17). Abundant conidia are produced on infested flowers (Plate VI., fig. 16) and this is important in the life history of this fungus, which does not produce spores readily on most media.

Flowers which are left in the field, thus, contribute a large proportion of the spore load of *Botrytis*.

This attack also causes considerable direct loss, through destruction of blooms.

Collar Rot.

The fungus occasionally causes collar rot of the growing plant under Victorian conditions. The foliage of the affected plants becomes yellow and finally the whole plant dies. When the affected plant is pulled up abundant sclerotes can be seen on the collar region of the plant (Plate VI., fig. 15).

This phase of the disease has not proved serious in Victoria, and is usually due to planting infected corms. It is apparently more important under English conditions (37).

The Causal Organism.

Isolation.

Numerous cultures from infected corms have been made, using tissue platings on to potato dextrose agar, and a species of *Botrytis* has been isolated consistently from the material.

Isolations have not been readily obtained from infected leaves. Moore (37) experienced the same difficulty. He explained this by suggesting that many of the spots represent abortive infections by *Botrytis*. However, *Botrytis* has been isolated from large leaf spots, formed by the coalescence of several smaller spots.

Isolations have been successfully made from infested flowers, by plating small portions of infected material, which had been surface sterilized with mercuric chloride, on to potato dextrose agar. Isolations have also been made from single spores on the flowers using Ezekiel's (17) modification of Keitt's method (31).

No difference could be detected between the characteristics of the organism isolated from the corms, leaves, and flowers.

Morphology.

Mycelium.—The mycelium of the fungus develops abundantly on infected corms held under moist conditions. It is white in colour and is loose and fluffy in texture. Mature hyphae are somewhat variable in size but average 12μ in diameter. Young hyphae are much narrower and average about 4 to 6μ in diameter. The growth on common artificial media is similar to the growth on the host. When grown on potato dextrose agar slopes, the mycelium develops profusely.

Sclerotes.—After several days' growth on the corm, or after about six days' growth on potato dextrose agar, the mycelium near the substrata darkens and sclerotes develop. These are at first creamy in colour but rapidly darken to black. Frequently many sclerotes coalesce forming large coralloid masses. Each individual sclerote is large in size and ranges from 1 mm. to 6 mm. in diameter. The surface of the sclerotes is smooth.

Macroconidia.—Macroconidia are not formed abundantly on artificial media, but develop abundantly on infected flowers, less abundantly on infected leaves and occasionally on infected corms. They are also produced from the sclerotes after several weeks storage under suitable conditions. The conidiophores are brown in colour and of the typical *Botrytis* type (Plate V., fig. 9). The conidiophores are about 12–14μ in diameter. The cells of the conidiophores are variable in length but average between 170–290μ. This is in marked contrast to the length of the cells of conidiophores of a strain of *Botrytis cinerea* isolated from lettuce. The cells of the conidiophores of the lettuce strain varied from 90–170μ. The macroconidia are ovoid in shape and are 13–18μ (average 15μ) long and 11–12μ (average 12μ) wide. They are thus considerably wider than the conidia of *Botrytis gladioli* as described by Klebahn (32), who gives the dimensions of conidia of that species as 8–15 $\times$ 3–6 (average 10·4 $\times$ 4·7μ). They agree with the dimensions of conidia from affected gladioli as given by Moore (37), who quotes the dimensions as 12–15 $\times$ 9–12μ (average 13 $\times$ 10μ), and with the dimensions given by B. O. Dodge and T. Laskaris (11), who give the dimensions as 12·5–21·4 $\times$ 8·3–13·2 (average 15·8 $\times$ 10·5μ).

However it is doubtful whether this difference is sufficient to regard this *Botrytis* as a different species to *Botrytis gladioli* Kleb.

Microconidia.—Microconidia were not observed on natural media, but develop freely in the depths of potato dextrose agar cultures over one month old. The sporodochia appear macroscopically as olivaceous green aggregations of hyphae. Microscopically the sporodochia are penicillate. This form is common to many *Botrytis* species (Drayton, 13). The microconidia are produced very abundantly and are spherical and about 2μ in diameter.

No "perfect" stage of the organism has yet been observed, but the development of microconidia suggests that a perfect stage exists. Drayton (13) states that, "it is highly probable that this sexual mechanism is operative, with perhaps slight modifications, in all of the spermatia-producing *Ascomycetes*, including in the term spermatia, microconidia of the type here described." Groves and Drayton (21) have shown the perfect stage of *Botrytis cinerea* is a *Sclerotinia*.

Physiology.

The organism grows freely on most common media, including potato dextrose agar and malt agar. Its growth on Czapek's solution is not vigorous, unless the solution is supplemented with vitamins. On all these media conidial production is sparse under ordinary conditions. Sclerotes are produced rapidly in cultures on artificial media if the organism has not been subcultured frequently. If, however, the organism is subcultured frequently it eventually ceases to form sclerotes in culture. The mycelium becomes yellowish in colour and a yellow pigment develops in the substratum.

This behaviour resembles the "dual phenomenon" described by H. N. Hansen and W. C. Snyder (23).

In a later note (24) the same authors describe the existence of two forms of *Penicillium notatum*. The C form is the normal conidial type which is maintained in that form if subcultures are made from conidia and care is taken to avoid carrying mycelium over during the transfer. If mycelium is used in subculturing the fungus reverts to the non-sporing M form, which produces a yellow pigment in the substratum.

As *Botrytis* sp. produces conidia sparsely on artificial media subcultures have always been made with mycelium, and this may explain the change in character of the fungus after prolonged subculturing. However, after repeated subculturing for over a year the organisms, although changed in appearance, was found to be still capable of infecting Gladioli.

The influence of a number of factors on the growth of the organism was investigated.

TEMPERATURE.

Methods.—The organism was grown in 200 cc. Erlenmeyer flasks on a liquid medium of potato extract and glucose solution prepared in the same proportions as potato dextrose agar. The solution was inoculated by adding a spore suspension of the organism. The conidia were developed on autoclaved Gladiolus flowers, which were inoculated from a recent isolate on P.D.A. and then exposed to sunlight on the laboratory bench.

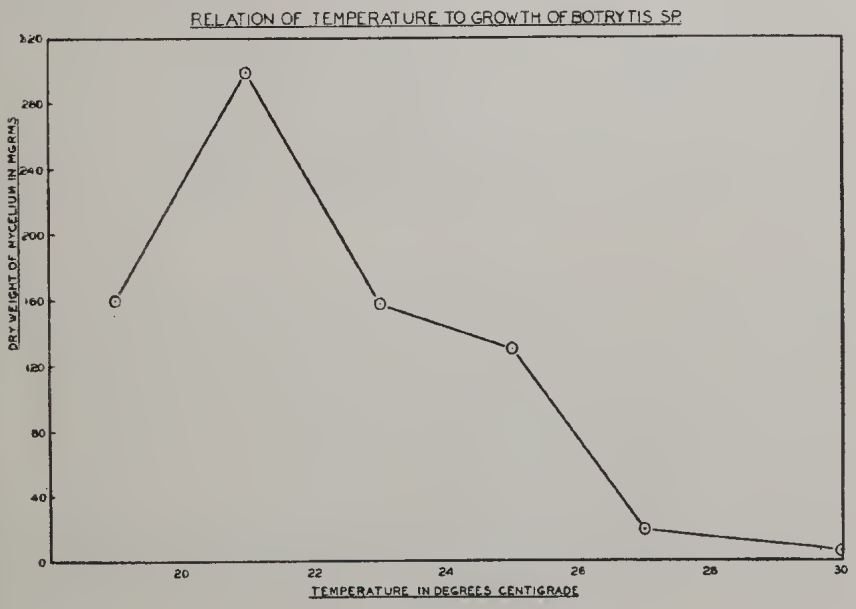
Six flasks were then incubated for ten days at each of the following temperatures:—19°C., 21°C., 23°C., 25°C., 27°C., and 30°C. The fungus mats were then filtered off, washed with hot water, dried at 105°C., and weighed.

Results.—The results are shown in Table I, and are illustrated in Text fig. 1:—

TABLE 1.

——	19° C.	21° C.	23° C.	25° C.	27° C.	30° C.
Weight of mycelium ..	grms. ·160	grms. ·299	grms. ·157	grms. ·130	grms. ·018	grms. ·006

The organism thus has an optimum temperature of about 21°C. and a maximum temperature of about 30°C., which is unusually low for most common fungi.



This low optimal temperature is in agreement with field observations that the disease becomes most serious late in the season when the temperatures are low and the humidity high.

Low temperatures favour other members of this genus. L. E. Hawker (25) has reported that *Botrytis narcissicola* Kleb. causes greater loss of Narcissus at cool or moderate, rather than at higher temperatures.

Brooks and Cooley (3) found that *Botrytis cinerea* had an optimum temperature of about 25°C. The amount of growth fell rapidly as the temperature was raised above the optimum and only slight growth occurred at 30°C.

Light.

Light has been found to stimulate the spore production of many fungi. Hall (22) found that light stimulated the sporulation of *Sclerotinia fructigena* and, when cultures on agar media in petri dishes were left exposed to sunlight on the laboratory bench, alternate zones of vigorously sporing mycelium and sparsely sporing mycelium were produced. The vigorously sporing zones were produced during the daytime and the sparsely sporing zones at night.

G. H. Coons (7) found that pycnidia of *Plenodomus fuscomaculans* were only produced in the presence of light, and C. Ternetz (52) found the asci of *Ascophanus carneus* were produced under the influence of light.

A. Beaumont *et al* (2) found that conidia of *Botrytis tulipae* developed on the host less rapidly in low than high light intensities.

W. Reidemeister (45) found that blue light, but not red light favoured the development of conidia of *Botrytis cinerea.*

Method A.—Cultures of the *Botrytis* on P.D.A. in petri dishes were prepared and when the growth was well established, half the dishes were removed from the incubator and placed on the laboratory bench.

Results.—Some conidia developed on the plates exposed to the light, particularly at the edges of the cultures, while no conidia developed on the unexposed plates in this experiment, and they have only rarely been observed on cultures which have not been exposed to the light. However, sporulation was not vigorous, even on the exposed plates, and therefore light is not the only factor which induces sporulation in the field.

A. Beaumont *et al* (2) produced conidia of *Botrytis tulipae* by exposing P.D.A. plates to light.

Method B.—Wheat grains were soaked over night in water and then 24 test tubes were half filled with the grains, plugged and autoclaved. They were then inoculated with *Botrytis sp.* Twelve

of the tubes were placed on the laboratory bench and twelve in an incubator with a glass front held at 23°C. Half of the tubes on the bench and half of the tubes in the incubator, were wrapped in brown paper to exclude light.

Results.—The tubes were examined after fourteen days incubation and it was found that the cultures exposed to the light on the bench and in the incubator had developed vigorously, and abundant sclerotes had been produced. In both cases the cultures from which light had been excluded, had only developed sparse mycelial growth and very few, small sclerotes. The results are illustrated in Plate VI., figs. 19-22.

Nicolaisen, W., *et al* (39), found that *Sclerotinia trifoliorum* behaves similarly and that darkness retarded both the mycelial and sclerotial development of that fungus.

NUTRITION.

Experiments have been conducted to determine the effect of increased carbohydrate and protein, and the presence of vitamins on the growth of the fungus. In preliminary experiments agar media were used and the diameter of the colonies determined as the criterion of growth. This method is not entirely satisfactory for no allowance is made for the density of the growth of the colony.

Method.—Potato dextrose agar was prepared containing ½ per cent., 1 per cent., 2 per cent., and 4 per cent. of dextrose. To P.D.A. of each of these dextrose contents, 0 per cent., ½ per cent., 1 per cent., and 2 per cent. of peptone were added. Fifteen cc. of the media were then poured into 10 cm. petri dishes and inoculated at the centre with mycelium of *Botrytis sp.* A uniform amount of inoculum was added by using a "biscuit cutter" 1 mm. in diameter as described by Keitt (31). The plates were then incubated at 23°C. in the absence of light. After nine days incubation the plates were examined and the diameter of the colonies determined. The experiment was conducted in quadruplicate.

Results.—The results are set out in Table 2, where the mean colony diameters are quoted:—

TABLE 2.

Peptone Concentration.	Dextrose Concentration.			
	.5 per cent.	1 per cent.	2 per cent.	4 per cent.
0 per cent.	5 cm.	7 cm.	7·5 cm.	10 cm.
½ per cent.	4 cm.	6·5 cm.	7·0 cm.	10 cm.
1 per cent.	4 cm.	6·0 cm.	7·5 cm.	10 cm.
2 per cent.	5 cm.	6·0 cm.	6·5 cm.	10 cm.

The most significant result of this experiment was the marked response to increase of dextrose concentration. A similar response by *Botrytis cinerea* has been found by J. L. Weimer and L. L. Hartner (56). They found that the dry weight of the mycelium increased with increased concentration of dextrose up to 30 per cent. dextrose, and was then reduced by further increase in dextrose concentration.

Peptone had no observable effect on the growth of the organism and apparently *Botrytis* sp. does not require large amounts of protein for growth.

Utilization of Various Sources of Nitrogen.

Method.—Czapek's solution was prepared with the usual formula of—

Magnesium sulphate	0·5 grms.
Potassium phosphate (K_2HPO_4) ..	1·0 grms.
Potassium chloride	0·5 grms.
Sucrose	30·0 grms.
Water	1000·0 ml.

The solution was then divided into six portions. No nitrogen was added to one series and 0·2 per cent. of sodium nitrate was added to another portion, 0·123 per cent. ammonium chloride, 0·163 per cent. of sodium nitrite, 0·156 per cent. of asparagin, and 0·177 per cent. of glycine respectively, were added to the other four portions, the nitrogen added being thus equivalent to 0·2 per cent. of sodium nitrate. 1·7 per cent. of agar was then added to each solution and, after autoclaving, 15 cc. of the various media were poured into sterile petri dishes. They were inoculated as previously described and then incubated at 23°C. for six days, when they were examined and the diameter of the colonies determined. There were four replicates of each treatment.

Results.—The results are shown in Table 3:—

Table 3.

Nitrogen Source.	Colony Diameter.	Type of Growth.
	cm.	
Nitrogen free	10	Extremely sparse growth
Sodium nitrate	10	Normal growth
Ammonium chloride	8	Normal growth
Sodium nitrite	4	Flat yellowish growth
Asparagin	7	Normal growth
Glycine	7	Normal growth

These results indicated that sodium nitrate was the most suitable source of nitrogen for *Botrytis* sp. S. J. Du Plessis (15) in experiments on the physiology of *Botrytis cinerea,* found that the greatest weight of mycelium per unit of nitrogen consumed was on a nitrate containing media.

Therefore sodium nitrate has been used as the nitrogen source in subsequent experiments.

These results demonstrate the unsatisfactory nature of colony diameter as a criterion, since the diameter of the colonies on media with no nitrogen was greater than when ammonium chloride, sodium nitrite, asparagin, or glycine were present, but actually the growth was extremely sparse and the weight of the colony would have been very much less than the weight of the colonies on any of the other media.

Utilization of Carbohydrate in a Synthetic Medium.

Method.—Czapek's solution plus agar, containing 1·5 per cent., 3 per cent., 6 per cent., and 12 per cent. of sucrose was prepared. The same technique as has been already described was used and the colonies were measured after six days' incubation.

Results.—The results are shown in Table 4:—

Table 4.

Sucrose Concentration.	Diameter of Colony.
%	cm.
1·5	3·0
3·0	2·5
6·0	3·0
12·0	4·0

Thus the fungus did not respond to increased sucrose concentration. It was therefore obvious that potato extract contained some growth factor not present in the synthetic medium, and without this factor the fungus did not respond to an increase in carbohydrate.

Effect of Vitamins on the Growth of the Fungus.

In 1858 Pasteur (43) had shown that growth of lactic acid bacteria was stimulated by the addition of onion juice to the medium. In 1860 Pasteur (44) found that the development of yeast in a synthetic medium was markedly improved by the addition of organic substances present in natural materials. These observations of Pasteur were the first indication of the existence of growth factors.

The importance of vitamins for the growth of certain fungi was first demonstrated by Schopfer (47) in 1934. He found that *Phycomyces Blakesleeanus* required thiamin for growth.

Schopfer (48) lists the following ascomycetes requiring thiamin for growth:—*Saccharomyces cerivisеae, Nematospora gossypii, Nectria coccinea, Sphaerula trifolii, Valsa pini, Helvella*

infula, and *Haplodermium pinestri*. The importance of thiamin for these organisms was demonstrated by a number of workers, who are quoted by Schopfer.

The importance of pantothenic acid was found by Williams *et al* in 1933 (58), and nicotinic acid was shown to be essential for the growth of *Staphylococcus aureus* by Knight (33) in 1935.

Biotin was extracted from egg yolk by Kögl and Tönnis (34) in 1936 and found to be still active on *Saccharomyces* at a dilution of 1 in $4 \times 10''$.

In 1939 Orla-Jensen *et al* (41) showed that lactic acid bacteria required riboflavin for growth.

A full account of the historical development of this subject is given by Schopfer (48).

In view of the known importance of vitamins to fungal growth, an experiment was conducted to determine whether a mixture vitamins of the B complex, either with or without biotin, would supply the factor, without which *Botrytis* did not respond to increasing dextrose concentration.

Methods.—Czapek's solution, with the addition of 0·5 grams of calcium chloride, but without sucrose was used as the base solution. Heavy metals (iron, copper, manganese, and zinc) were added to the base solution. Solutions containing 0·5 per cent., 1 per cent., and 2 per cent. of dextrose were prepared.

Ten per cent. by volume of potato extract, prepared by boiling 20 grams of potato in 100 ml. of water and filtering, was added to one series.

Members of the B complex of vitamins (thiamin, riboflavin, nicotinic acid, calcium pantothenate, and pyridoxin) were added to another series to give final concentrations of 1γ per 50 ml.

These vitamins, together with biotin concentrate to give a final concentration of 1γ per 50 ml. were added to another series. No addition was made to a fourth series of solutions.

Forty-eight ml. of the various solutions were pipetted into 200 ml. Erlenmeyer flasks and autoclaved at half an atmosphere for twenty minutes.

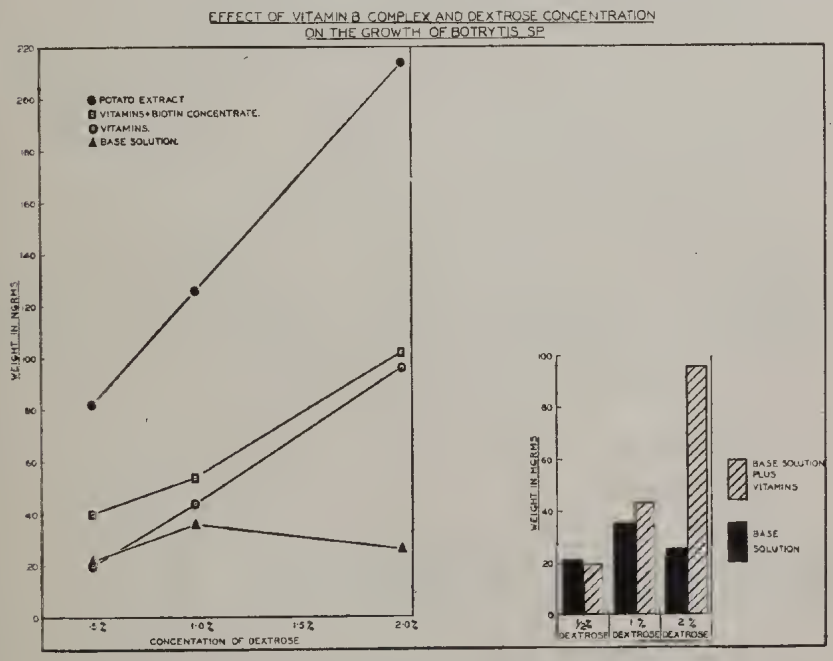
They were then inoculated by adding 2 ml. of a spore suspension of *Botrytis* with a sterile pipette. The spore suspension was prepared from a culture on autoclaved gladiolus flowers. To reduce the risk of carrying over vitamins from the flowers, the spores were washed twice by centrifuging, syphoning off the supernatant liquid with sterile capillary tubing, and adding fresh distilled water.

The flasks were then incubated at 25°C. for ten days, when the mycelium was filtered off, washed with boiling water, dried at 105°C., and weighed.

Results.—The results are shown in Table 5 and are presented graphically in fig. 2:—

TABLE 5.

	Dextrose Concentration.		
Addition to base solution	% ½	% 1	% 2
No addition	·022	·036	·26
B. complex vitamins	·020	·044	·096
B. complex vitamins plus biotin conc.	·040	·054	·102
Potato extract	·082	·126	·214



The following statistical report on the results has been prepared by Dr. H. C. Forster of the Victorian Department of Agriculture:—

"A statistical analysis of the results has been conducted to determine (*a*) the significance of the difference between the respective treatments, (*b*) the significance of the difference, if any, between the regression coefficients of the three treatments which included vitamins.

Since the variation shown by the replicates of the different treatments varies directly with the mean value of the treatment, the analysis has been conducted not on the original figures but on a transformation based on log $(100\ x)$.

COMPARISON OF THE DIFFERENT TREATMENTS.—RESULTS ON BASIS OF LOG $(100\ x)$.

Base Soln.			Base + VB.			Base + VB + biotin.			Base + Potato Extract.		
½%	1%	2%	½%	1%	2%	½%	1%	2%	½%	1%	2%
·34	·54	·40	·30	·64	·98	·60	·68	1·08	·92	1·10	1·34
·43			·64			·79			1·20		

S.E. (between dosages) = ·054 For significance, differences must exceed ·15.
S.E. (between treatments) = ·033 For significance, differences must exceed ·09.
F 1% both between main treatments and within main treatments.

It is evident therefore that there are significant differences both between the various main treatments, and except in the case of the base solution, between the dosages of those treatments.

COMPARISON OF REGRESSION COEFFICIENTS OF DOSAGE REGRESSION LINES OF THE VARIOUS TREATMENTS.

It is obvious that the regression line of the base solution is significantly different from the other three regression lines. The base solution treatment has therefore been deleted from this analysis and a test conducted to see whether the other three regression lines differed one from the other.

Analysis. Treatment d/f.	x.	y.	xy.	"b" $= \frac{\Sigma xy}{\Sigma x^2}$	Errors of Estimate. $y - \frac{(\Sigma xy)^2}{\Sigma x^2}$	*d/f.*	*M.S.*
Base + VB 2	·90	1·156	1·020	1·133	0·004	1	
Base + VB + biotin 2 ..	·90	·662	0·720	0·800	0·086	1	
Base + Potato Extract 2 ..	·90	·448	0·630	0·700	0·007	1	
					0·097	3	0·032
Total	2·70	2·266	2·370	0·878	0·185	5	0·037

$F = \frac{·037}{·032}$ which is not significant.

It is apparent therefore that there is no significant difference between the regression coefficients of these three treatments.

It should be noted that the regression coefficients of the above table are those of the transformed figures. (References—Snedecor (51) and Cochran (6).)

The results demonstrate that there is no response to increasing dextrose concentration when no vitamins and a purely synthetic medium is used. When vitamins are added to the base solution however, a marked response is obtained. There was no significant difference between the gradients of the curves for increasing dextrose content when vitamins and biotin, or potato extract are added to the base solution. This suggests that vitamins are the main limiting factor in preventing response to increased dextrose. The greater growth obtained with potato extract could be explained by the additional carbohydrate added with the extract and an extra growth factor is not necessarily present.

Schopfer (48) in commenting on results obtained by Leonian and Lilly, suggests that the effect of organic acids (succinic and fumaric) on the response of *Phycomyces* to thiamin is due to the addition of nutrients (carbon compounds and minerals) rather than to an additional growth factor. In this case the response could not be due to minerals, since pure mineral salts were not used and all the elements known to be important in fungal nutrition were added to all solutions.

Little response to vitamins was obtained at low dextrose concentrations, but a very marked response when 2 per cent. was present (see fig. 2). Burkholder and McVeigh (4) found that with 4·0 and 8·0 grams per litre of asparagin and thiamin at 1×10^{-6} Molar, glucose was limiting up to quantities of 80 or 100 grams per litre.

A definite response to biotin concentrate was obtained. Crystalline biotin was not available for the experiment and unpublished data by Millikan suggests that the biotin concentrate used contains growth factors other than biotin.

It is unlikely that all the vitamins added are essential for the fungus and further work will be conducted to elucidate this point.

Production of Conidia.

In the field, conidia are produced abundantly on infected flowers, less abundantly on infected leaves and occasionally on infected corms, but, as already stated in previous sections, conidia are not produced readily on ordinary media.

Newton (38) found that conidia of *Botrytis tulipae* were produced when the fungus was grown on tulip extract agar, although this fungus does not produce conidia on barley meal, corn meal, or synthetic agar media. Gladiolus dextrose agar was prepared in the same manner as potato dextrose agar. However, when the fungus was grown on this medium, in the absence of light, in an incubator at 23°C., no conidia were produced. When grown on this medium in the presence of light on the laboratory bench some conidia were produced, but not more than on potato dextrose agar under the same conditions.

As conidia are produced on naturally infected flowers, gladiolus flowers were placed in Erlenmeyer flasks and autoclaved. They were then inoculated with *Botrytis* sp. and placed in an incubator at 23°C. They were then transferred to a laboratory bench exposed to the light. In 21 days after the start of the experiment, conidia had developed abundantly on the flowers.

Hopkins (28) noted the vigorous sporulation of *Botrytis tulipae* on infected flowers and suggested this was due to the favorable moisture relations. He therefore grew *Botrytis tulipae* on potato dextrose agar in petri dishes and allowed the medium to dry out and conidia were produced on these plates.

Reidemeister (45) considered that the drying out of cultures, or culturing on media of high osmotic pressure, were the most important factors in inducing sporulation of *Botrytis cinerea.*

An experiment was conducted to determine whether drying out of the medium or exhaustion of the food supply would induce sporulation of *Botrytis* sp.

Method.—One millilitre of potato dextrose agar was pipetted into each of twelve 200 ml. Erlenmeyer flasks, autoclaved and inoculated with *Botrytis.* They were then incubated at 23°C. for seven days. The plugs of six flasks were then dipped into paraffin to prevent the drying out of the agar. The remainder were unwaxed. Three waxed and three unwaxed flasks were then placed on the laboratory bench, where they were exposed to light, and the same number of waxed and unwaxed flasks left in the incubator away from light.

Results.—After fourteen days it was found that conidia had developed on both sets of flasks which were exposed to light, but no conidia had developed on either set kept away from light.

A similar experiment was also conducted with gladiolus flowers, gladiolus stems and cyclamen flowers, and again it was found that conidia were produced on the materials in both waxed and unwaxed flasks, which were exposed to light, but not in the flasks kept away from light.

These results indicate that both exhaustion of the food supply and drying out of the medium can induce sporulation provided the cultures are grown in the presence of light..

The effect of impoverishment of food supply on sporulation has been observed with many fungi. For example, G. H. Coons (7) found that rapid fruiting of *Plenodomus fuscomaculans* could be induced by removing a strongly growing culture to a dilute nutrient solution, or to distilled water. It appears that factors which are unfavorable to continued vegetative growth, are favorable to sporulation.

It has been noted that, after preparing a spore suspension from spore-bearing flowers in Erlenmeyer flasks, sporulation is profuse. This may be due to washing away nutrients when the suspension is prepared.

Apart from sporulation which develops from the mycelium under the conditions described, conidia are produced from sclerotes on cultures on any common medium, after the cultures are several months old. Plate V., fig. 11, shows conidial production from a sclerote from a potato dextrose agar culture. Conidial production has been observed from sclerotes in cultures which had been stored away from light, but conidial production occurs more rapidly in cultures exposed to light.

Botrytis cinerea produces conidia from sclerotes in a similar manner (60).

Effect of the Fungus on the Corm.

Histology.

Sections were cut of corms in which the disease was active and of corms in which the disease had been arrested. The usual method of paraffin embedding was used, except that 5 per cent. of microcrystalline wax was incorporated in hard paraffin to prevent the paraffin forming large crystals and thus facilitate sectioning.

In active lesions the middle lamella of the cells of diseased parenchymatous tissue had been destroyed and the cells greatly distorted. The cell contents showed no definite structure but contained an accumulation of starch granules. At the edge of diseased lesions there is usually a sharp line of demarkation between the almost completely disorganized diseased tissue and the surrounding normal tissue (Plate VII., fig. 23). The mycelium of the fungus was abundant in the disorganized tissue and sometimes penetrated to a depth of several cells into apparently normal tissue.

In some sections, however, there was a layer of cells containing a reduced number of starch grains, between the infected tissue and the normal tissue (Plate VII., fig. 24). The infected tissue contained an accumulation of starch granules and there was no suberinised layer at the edge of the healthy tissue. It therefore differed from the histological structure of arrested lesions, which will be described later.

As previously stated the disease travels along the vascular bundles. The phloem tissue of the infected bundles is rapidly disintegrated and later the wood vessels are attacked and destroyed. Plate VII., figs. 26*a* and *b* shows a longitudinal section through an infected vascular bundle. The phloem tissue has been

almost completely destroyed but the wood vessels still show fairly normal structure. Hopkins (28) noted that *Botrytis tulipae* destroyed the xylem of infected tulips.

Infected tissue of corms, in which the disease has been arrested differ in several respects from those just described. The severely infected tissue is similar to that in actively growing lesions but no starch granules are present. It is surrounded by a layer of cells about 1 to 2 mm. wide, which have practically no cell contents and very few starch grains, but the cell walls do not show marked distortion. This layer of cells only contains few hyphae of the organism. At the edge of these cells there is a layer of rectangular suberinised cells and beyond that the tissue is normal (Plate VII., fig. 25).

The development of a suberinised layer around diseased lesions in tubers and corms has frequently been reported. Hill and Orton (27) found that potato tubers infected with bluestem disease produce a layer of suberinised tissue around the infected tissue.

Microchemical Tests.

To determine the chemical changes in the diseased tissue a series of microchemical tests were conducted. Fresh, hand sections were used, and methods described by Johansen (30) and by Hill and Orton (27) were employed for most of the tests.

Methods and Reagents Used.

Starch and Dextrin.

The usual iodine test.

Suberin.

A solution of Sudan III. in 95% alcohol.

Reducing Sugars.

The osazone test as described by Johansen.

Proteins.

The sections were stained for 24 hours in a saturated aqueous solution of picric acid.

Cellulose.

They were placed in a drop of iodine solution and a drop of 75% sulphuric acid was allowed to diffuse under the coverslip.

Methyl Pentoses.

The sections were placed in one or two drops of acetone, a drop of hydrochloric acid was added and the sections warmed for fifteen minutes.

Lignin.

The sections were placed in a 1% alcoholic solution of phloroglucinal and a drop of hydrochloric acid added.

ARABAN AND XYLAN.

The test was conducted as for lignin but the sections were warmed for ten minutes.

PECTIN.

The sections were stained in a dilute aqueous solution of ruthenium red.

TANNINS.

The sections were placed in 10% aqueous ferric chloride plus a little sodium carbonate.

SAPONINS.

The sections were placed in concentrated sulphuric acid.

RESINS.

The sections were left in a 7% aqueous solution of copper acetate for 5 days.

OXIDASE.

The sections were placed in a 1% solution of benzidine in 60% alcohol.

PEROXIDASE.

The sections were placed in a 1% solution of benzidine in 60% alcohol and a drop of hydrogen peroxide added.

CATALASE.

The sections were placed in a 1% solution of gum arabic and a drop of hydrogen peroxide was added.

NITRATES.

The sections were placed in a 0·1% solution of diphenylamine in 75% sulphuric acid.

PHOSPHATES.

The method used was that employed by Humphrey and Dufrenoy (29). The sections were placed in a mixture of 5 ml. of a solution of 20·8 ml. of sulphuric acid, and 6·41 grms. of ammonium molybdate made up to 250 ml. with distilled water; and 1 ml. of a solution of 0·5 grms. of 1-amino-2-naphthol-4-sulphonic acid and 5·75 grms. of sodium-bisulphite, plus 5 ml. of a 20% solution of sodium sulphite made up to 90 ml. with distilled water.

CALCIUM.

The sections were placed in a 2% aqueous solution of oxalic acid. The acid was withdrawn after thirty minutes, a coverslip added and alcohol allowed to diffuse under the coverslip.

CALCIUM OXALATE.

The sections were placed in a 7% aqueous solution of copper acetate.

SULPHATES.

The sections were placed in a 1% solution of benzidine chloride in 3% hydrochloric acid.

Results.—The parenchymatous tissues of healthy gladiolus corms contain starch grains, but they are not present in the tissue of the vascular bundles. In corms in which the disease is active,

excessive accumulation of starch occurs in the infected tissue (Plate VII., figs. 23 and 24). The surrounding healthy cells do not show any marked reduction in the number of starch grains present. Hopkins (28) noted that starch accumulated in tulip bulb tissue infected with *Botrytis tulipae*.

Pectin is absent from the diseased areas but is present as the middle lamella in healthy tissue. The capacity of *Botrytis* species to utilize pectin has been noted by several workers. A report by the Food and Vegetables Committee, Department of Science and Industrial Research (9) states that in studies of the parasitism of *Botrytis* sp. on the apple it was found that the organism utilized considerable quantities of pectin. Davidson and Willaman (8) reported that *Botrytis cinerea* produces pectinase. The capacity of *Botrytis* to utilize pectin explains the rapid disorganization of infected tissue.

The cell walls are changed to a material which stains yellow with iodine and is apparently a dextrin.

No reducing sugars were detected in healthy tissue, but glucosozones developed in some sections of diseased tissue which were tested. The osozones did not appear till after forty-eight hours, which suggests the reducing sugar present was glucose.

Saponins and the enzymes catalase and peroxidase were present in both healthy and diseased tissue. Oxidase was not detected in diseased tissue and it was only detected in developing shoots of the healthy tissue.

Free nitrates, lignin, methyl pentoses, resins, tannins, calcium, calcium oxalate, and sulphates were not detected in either healthy or diseased tissue. Suberin was not detected in, or at the edge of, active diseased lesions. The phosphate test was not conducted on material of this type. No deposits of protein material were detected in healthy or diseased tissue.

A pigment, which changes to vinaceous-rufous—Ridgeway's colour chart (46)—on the addition of alkali is produced in infected tissue. This pigment is water soluble and, when extracted from the corm, is amber yellow coloured. It changes to vinaceous-rufous at pH 6·8 and may be precipitated from aqueous solution by the addition of excess acid. The chemical nature of this material has not been determined.

Small quantities of the same pigment are produced in gladiolus corms infected with *Septoria gladioli* or *Bacterium marginatum*.

The pigment is not produced by *Botrytis* when the organism grows on potato dextrose agar or gladiolus dextrose agar. *Botrytis* was grown on autoclaved gladiolus corms, but no

indicator pigment was produced. Therefore the pigment is produced by living gladiolus corm tissue when invaded by *Botrytis, Septoria gladioli,* or *Bacterium marginatum.*

Kreuzer (35) found that a pigment which changes in colour from red at pH 8·5 to yellow brown at pH 4·5, is produced in onion roots infected with *Phoma terrestris.*

The chemistry of infected tissue in corms in which the disease has been arrested differs in several features from infected tissue of corms in which the disease is active. The disorganized tissue contains little or no starch but is similar in other respects to that described previously.

The phosphate test demonstrated the presence of free phosphate, or loosely combined phosphorous compounds, in the healthy tissue, but no reaction for phosphates was obtained in diseased tissue. Humphrey and Dufrenoy (29) found that free phosphate appears in oat tissue infected with crown rust. Apparently in the case of *Botrytis* the phosphates are used by the fungus and do not accumulate. This tissue is surrounded by a layer of cells, about 1 to 2 millimetres wide, which is practically devoid of cell contents. The cell walls are not distorted in shape but stain yellow with iodine showing that they have been partially broken down to dextrins. The middle lamella stains progressively fainter with ruthenium red towards the disorganized tissue, indicating that it has been partly dissolved.

This layer is surrounded by rectangular cells, with suberinised walls, and beyond the tissue is normal (Plate VII., fig. 25).

Pathogenicity and Host Range.

The pathogenicity of *Botrytis* to gladioli was demonstrated by inoculating healthy corms, with a pure culture of the organism. The organism was introduced by needle puncture, and the corms were then placed in jars containing a free water surface. After several days brown lesions, typical of the disease in the field, developed (Plate V., fig. 12) and they increased rapidly in size with continued incubation.

Isolates were made from the edges of lesions on artificially infected corms, and *Botrytis* was consistently isolated.

The disease has only been observed under field conditions on varieties of *Gladiolus primulinus* and gladiolus hybrids.

Limited infection experiments, using the technique described above, were conducted on corms or bulbs of cyclamen, narcissus, *Gladiolus colvillii,* and *Ixia grandiflora.* Infection occurred in corms of *Gladiolus colvillii* and *Ixia grandiflora,* though the disease has not been observed on these plants in nature.

Method of Infection in the Field.

It has previously been stated that *Botrytis* enters the gladiolus corm along the vascular bundles. Large numbers of infected corms were examined and in all cases at least portion of the core of the corm was infected and had spread from the core to other portions of the corm along the vascular bundles. In no case had the fungus entered the corms through parenchymatous tissue.

From theoretical considerations the fungus could enter the core of the corm through the old corm from infected soil, it could pass down into the corm from infected foliage or it could enter the corm by infection of the cut stem, or the old corm, after the corms were dug.

In most of the infected corms examined the whole of the core was diseased. In some corms, however, only the top portion of the core was infected. In these cases infection could have occurred by the fungus passing down into the corms from infected leaves or by infection of the cut stem end while the corms were on the drying racks (Plate V., fig. 8). A few corms only showed infection of the lower portion of the core. Infection could have occurred from infected soil or by infection of the corms on the drying racks.

Pot experiments were conducted in 1941-42 in order to determine the probable method of infection in the field.

The susceptible variety, Picardy, was used for the experiment and the plants were grown in virgin, red mountain soil in 8-in. pots. One series was planted into soil which was inoculated by mixing it with infected corm material. The leaves of another series were inoculated, before the plants flowered, by brushing them with conidia developed on artificially infected flowers. Another two series were inoculated in the same way immediately after flowering. After inoculation the plants were held in a humidity chamber for twenty-four hours and then removed to the glass-house.

Abundant lesions, typical of *Botrytis* infection in the field, developed on all the inoculated leaves.

The corms were dug six weeks after flowering and the pre-flowering inoculated series, one of the post-flowering inoculated series and the soil inoculated series were stored in closed tins to reduce the rate of drying of the corms. The other post-flowering inoculated series was stored under good conditions on a wire-netting stretcher.

The freshly cut stem ends of two other series were inoculated by brushing with dry conidia of *Botrytis*. One series was stored in a closed tin and the other on a wire-netting stretcher.

Two other series were not inoculated and one stored in a closed tin and the other on a wire-netting stretcher.

There were six replicates in each series throughout the whole experiment.

After storage for eight weeks the corms were examined and it was found that typical *Botrytis* rot had developed in the series in which the cut ends of the corms were inoculated at digging time and then stored in closed tins. The corms in all the other series did not develop the disease.

This experiment demonstrated that infection can occur through the cut stem ends of the corms, if they are stored under humid conditions. The experimental conditions may not have been favorable for soil infection as the pots were well drained, and soil infection may require a high soil moisture content. However, evidence in the field does not suggest soil infection is important, and serious infection of corms, which were grown on virgin soil, has been observed. The failure of soil sterilization experiments to control the disease supports this view.

As heavy leaf infection was obtained in the experiment, it is unlikely that infection of corms develops by the disease passing down from the leaves into the corms. This view is supported by the failure of foliage sprays to control the disease, and evidence from the control experiments conducted subsequent to this experiment suggests that all field infection occurs on the drying racks after digging.

As some of the infection on the racks could occur through the cut stem end of the corm and some through the old corm it seemed possible that the amount of infection would be reduced if the tops were not removed from the corms after digging.

Therefore an experiment was conducted in the 1943-44 season in which 200 corms of Picardy were dug and stored, without removing the tops, on wire-netting stretchers. The tops were removed from an equal number of Picardy corms and they were stored under the same conditions.

The corms were examined after ten weeks' storage and it was found that 45 per cent. of the corms from which the tops had been removed were infected, while only 34 per cent. of the other series were infected. Thus the disease enters both from the cut stem end and through the old corm.

Control.

Control measures recommended by Dodge and Laskaris (11) and Moore (37) are stringent field and storehouse sanitation. These methods would probably reduce the disease but are not a completely satisfactory solution to the problem.

The experiments on control described here were designed to prevent the entry of the fungus into the corms. As the disease attacks the interior of the corm it is obvious that dipping infected corms with fungicidal solutions will not control the disease. A limited number of experiments were carried out in an attempt to sterilize infected corms by volatile materials, and these will be described later, but the method was not successful.

Because of the three possible methods of entry described in the preceding section, experiments were conducted on soil sterilization, spraying to prevent foliage infection and dipping of the corms at digging time.

Experiments were also conducted on the influence of time of digging and to determine whether any varieties of the gladiolus were resistant to the disease.

Soil Sterilization Experiments.

An experiment on the effect of soil sterilization was conducted in the 1940-41 season.

Method.—Trenches 4 inches deep and 40 feet long were dug, and the fungicidal materials were then applied. The soil was then replaced and the treated areas covered with bags for five days. After fourteen days 100 corms of the variety Picardy were planted in each treated row and an untreated row. The experiment was laid out as a randomized block and there were four replicates of each treatment.

The treatments tested were—formalin, 2 per cent. applied at the rate of ½ gallon per square yard, bleaching powder at the rate of 1 lb. per 20 square yards and carbon bisulphide at the rate of 1 pint per square yard.

Six weeks after the plants had flowered they were dug and placed on wire-netting stretches in the same order as their position in the field. After storage for six weeks the scales were removed from the corms and the number infected with *Botrytis* were determined.

Results.—The plants grew normally, except that the foliage of the carbon bisulphide treated rows was deeper coloured than the controls during the early stages of growth, though the effect was not maintained throughout the season. This was apparently due to partial soil sterilization (Waksman, 55).

Table 6.

——	Control.	Formalin.	Bleaching Powder.	Carbon Bi-sulphide.
Percentage	29·6%	39·3%	26·3%	25·2%
Angle	32·94°	38·77°	30·86°	30·07°

Determination of the F value (Snedecor, 50) showed that the differences in the experiment were not significant. The method of angular transformation is taken from Cochran's paper (6).

Soil sterilization does not therefore appear to offer any possibility of control, and this experiment, considered together with evidence previously presented, indicates that soil infection is not an important factor in the disease. Therefore soil sterilization experiments were not conducted in subsequent seasons.

Spraying Experiments.

Methods.—Corms of the variety Picardy were planted in rows of 100 corms according to ordinary commercial practices. The experiments were sprayed in randomized blocks, each row of 100 corms constituting a single plot. There were four replicates of each treatment and the same number of unsprayed plots.

The plots were dug six weeks after flowering and placed on wire-netting stretchers in the same order as the position of the plots in the field. The scales were removed from the corms after six weeks' storage and the number of infected corms determined.

Results (1940-41 season).—Lime sulphur 1 in 40 and 6:4:40 Bordeaux mixture were tried in that season. Agral II. was added to the sprays at the rate of 1 in 2,000, and good wetting of the foliage was obtained. Spraying was commenced one week after flowering, as foliage infection does not occur before flowering under Kalorama conditions.

Lime sulphur was ineffective and lesions developed on the sprayed plants. Bordeaux mixture prevented foliage infection for a fortnight after application, but later some lesions developed as the spray washed off the leaves.

Table 7 shows the percentage of infected corms:—

Table 7.

——	Unsprayed.	Bordeaux.	Lime Sulphur.
Percentage	23%	26·8%	24·5%
Angle	28·66°	31·15°	29·68°

The F value was determined and it was shown that the differences were not significant. However, it was felt that this could have been due to an insufficient number of applications of Bordeaux, and therefore a further spraying experiment was conducted in the 1941-42 season.

In that season 6:4:40 Bordeaux mixture, plus 1 in 2,000 Agral II., was applied at weekly, fortnightly, and monthly intervals, copper oxychloride ("Soltosan") at the rate of 3 lb. in 40 gallons, plus 1 in 3,000 Agral II. at fortnightly intervals, and a commercial copper dust ("Coppodust") at fortnightly intervals, were tried.

The copper oxychloride caused slight foliage injury, and did not prevent leaf infection. Copper dust did not cause injury but was ineffective. Bordeaux was effective in reducing the number of leaf lesions in the plots that were sprayed at weekly and fortnightly intervals. It delayed infection in plots sprayed at monthly intervals but did not prevent its development before the corms were dug.

Table 8 shows the percentage of infected corms. Analysis by the F value showed that the differences were not significant:—

TABLE 8.

	Unsprayed.	Bordeaux Weekly.	Bordeaux Fortnightly.	Bordeaux Monthly.	Soltosan.	Coppodust.
Percentage ..	2·5%	8·4%	6·1%	4·0%	4·8%	4%
Angle	9·17°	10·83°	14·23°	11·46°	12·60°	12·07°

In this season the percentage of infected corms on the untreated plots was low, and the experiment was not therefore entirely conclusive. However, no evidence of possible control by spraying was obtained for the second successive year, and therefore spraying experiments were not conducted in subsequent seasons.

TREATING THE CORMS AT DIGGING TIME.

Dipping of corms, bulbs and tubers is usually conducted during the dormant period before planting, with the object of destroying diseases present on the surface of the corm. However, this would not be effective against the *Botrytis* disease of the gladiolus and therefore experiments were conducted to find a method of preventing entry of the disease into the corms.

L. Hawker (25) tried dipping narcissus corms in cold formalin at digging time but it did prevent infection with *Botrytis narcissicola*. She found, however, that cold formalin, brassisan, folosan, and ceresan reduced losses due to *Fusarium bulbigenum*. F. Weiss *et al* (57) found that mercury compounds, particularly ethyl mercuric chloride and ethyl mercuric phosphate, were effective in preventing *Fusarium* basal rot of narcissus. A two-minute dip was as effective as longer treatments. The treatment was most effective if given immediately after digging. They found that the treatments caused injury to the flower buds in

subsequent crops, and they did not achieve a practical compromise between effective control and no flower injury. A number of treatments at digging time have been tried against *Botrytis* of the gladiolus.

Methods.—After digging, the corms were washed free of soil with a water spray and then counted into batches of 100. They were then placed in light hessian bags and dipped in the fungicidal solutions. After draining they were then spread out on wire-netting stretchers and stored on racks for drying. There were four replicates of each treatment and they were arranged on the stretchers in a randomized block arrangement.

After storage for six weeks the scales were removed and the number of infected corms determined.

Results (1940-41 season).—Dipping treatments tried were lime sulphur 1 in 20 for six hours and copper sulphate 2 per cent. for 24 hours. Another series was dusted with penta-chloro-nitro-benzene ("Folosan"). The variety Wolfgang von Goethe was used for the experiments. Gram and Thomsen (20) found 2 per cent. copper sulphate effective in controlling *Botrytis tulipae*. Pentachloro-nitro-benzene was found by Smieton and Brown (49) to control *Botrytis cinerea* on lettuce.

TABLE 9.

—	Untreated.	Lime Sulphur.	P.c.n.b.	Copper Sulphate.
Percentage	4·5%	9·3%	2·0%	No count possible
Angle	12·27°	17·71°	8·00°	

The F value was determined and the differences found to be non significant.

Copper sulphate caused severe injury and no count of infected corms was possible.

Neither of the other treatments caused any significant reduction in the number of infected corms.

Results (1941-42 season).—In that season shorter dipping times were employed to guard against similar injury to that produced by the copper sulphate treatment. The variety Hindenburg's Memory was used for the experiment. 6:4:40 Bordeaux mixture, plus 1 in 2,000 Agral II., for half an hour; lime sulphur, 1 in 40, plus 1 in 2,000 Agral II., for half an hour; mercuric chloride (corrosive sublimate), 1 in 1,000 for 1 hour, and a commercial brand of copper dust "Coppodust" were tested.

Table 10 shows the number of infected corms:—

TABLE 10.

	Untreated.	Corrosive Sublimate.	Bordeaux.	Cu Dust.	Lime Sulphur.
Percentage	5·9%	0·6%	3·4%	3·2%	12·4%
Angle	14·02°	1·43°	10·70°	10·24°	20·66°

Whole experiment highly significant by F test.

Difference for significance at 1 per cent. level .. 3·48°
Difference for significance at 5 per cent. level .. 2·48°

The method of analysis of variance described by Snedecor (51) was used.

Both corrosive sublimate and copper dust produced a reduction in infected corms which was significant at the 1 per cent. level, though the control exercised by copper dust was not sufficient to be of much commercial value. Corrosive sublimate exercised good control but caused a superficial injury, in the form of a hard brown depression along the base of the scales on the corms. However, when these corms were grown the plants were normal and produced normal flowers. Bordeaux mixture caused a reduction in the number of infected corms which was significant at the 5 per cent. level, but it was not sufficient to be of commercial value.

The number of infected corms produced in the lime sulphur treated corms was significantly higher than the untreated. This was apparently due to the fact that the treatment delayed the drying of the corms and they remained susceptible for a longer period than untreated corms.

Results (1942-43 season).—In this season corrosive sublimate, 1 in 1,000, plus Agral II, 1 in 2,000, for one minute and for 30 minutes; and Hortosan D.P. at the rate of 1 oz. in 5 gallons for two minutes and fifteen minutes were tested. The corms were placed in bags of cheese-cloth instead of hessian bags. The variety Picardy was used for the experiment.

Hortosan D.P. is a proprietary material containing 2·5 per cent. of mercury as an organic mercuric compound.

TABLE 11.

	Untreated.	Corrosive Sublimate 1 minute.	Corrosive Sublimate 30 minutes.	"Hortosan" 2 minutes.	"Hortosan" 15 minutes.
Percentage ..	46·7%	4·1%	1·8%	6·0%	0·6%
Angle	43·13°	11·64°	12·64°	14·12°	1·43°

The whole experiment was shown to be highly significant by the F test.

Difference for significance at 1 per cent. level .. 5·85°
Difference for significance at 5 per cent. level .. 4·07°

All treatments caused a significant reduction in the number of infected corms. Hortosan D.P. for fifteen minutes was significantly better than all other treatments. There was no significant difference between the two corrosive sublimate treatments. No injury was caused by any of the treatments.

Results (1943-44 season).—The primary object of the experiment in this season was to test the effectiveness of other proprietary organic mercurials, and to repeat tests on Hortosan D.P. for fifteen minutes and corrosive sublimate, plus 1 in 2,000 Agral II., for two minutes. Aretan (containing 3·5 per cent. mercury as methyl-oxy-ethyl-mercuric chloride used at the rate of 4 oz. in 10 gallons) and Zetan (synonymous with New Improved Semesan Bel., containing 12 per cent. hydroxy mercuric nitro phenol and used at the rate of 1 lb. to 10 gallons), were also tested. A dipping time of fifteen minutes was used for both materials.

The variety Picardy was used for the experiments and the corms were dipped in light hessian bags.

TABLE 12.

	Untreated.	"Hortosan" D.P.	"Zetan."	"Aretan."	Corrosive Sublimate.
Percentage ..	47·5%	8·4%	10·5%	16·9%	15·6%
Angle	43·55°	16·84°	18·89°	24·27°	23·23°

Whole experiment hightly significant at 1 per cent. level.

Difference for significance at 1 per cent. level .. 8·31°

Difference for significance at 5 per cent. level .. 5·93°

All treatments caused a significant reduction in the number of infected corms. There was no significant difference between treatments at the 1 per cent. level, but at the 5 per cent. level Hortosan D.P. was significantly better than Aretan or Corrosive sublimate.

REPLENISHMENT OF SOLUTION.

From an economic point of view it would be desirable to be able to use the same solution for several batches of corms. It is obvious that some reduction in strength of the dipping solution will occur after each batch of corms. Gilmore and Robinson (18, 19) investigated the loss in strength of corrosive sublimate solutions after dipping seed potatoes.

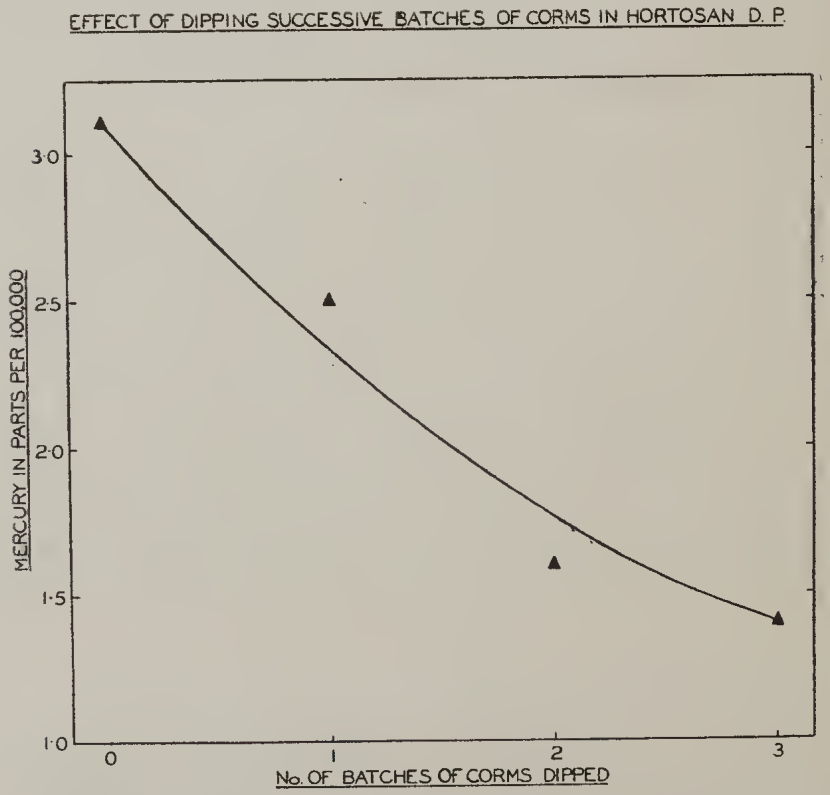
Therefore in 1944 a dipping experiment was conducted to determine the loss in strength of Hortosan D.P.

Method.—Four gallons of Hortosan D.P. solution was prepared and a sample of the solution taken. Then three successive batches of 200 washed corms were dipped in light hessian bags. Each dipping was for exactly fifteen minutes. After each dipping, samples of the solution were taken. The mercury content of the solutions was determined by Mr. W. Jewell, Agricultural Research Chemist of the Victorian Department of Agriculture.

Results.—The results are shown in Table 13 and are presented graphically in Text fig. 3:—

TABLE 13.

	Original.	After One Batch of Corms.	After Two Batches of Corms.	After Three Batches of Corms.
Mercury in parts per 100,000	3·1	2·5	1·6	1·4



In the figure a curve has been fitted to the points. It is not surprising that the points do not fall exactly on the curve, since variability would be expected because of variation in the size of the corms and the amount of soil on the corms.

Gilmore and Robinson (18) have pointed out a similar variability when dipping potato tubers in corrosive sublimate. Because of this factor they developed a field method (19) for determining the amount of corrosive sublimate necessary to replenish the solution.

Unfortunately, no similar method could be developed for organic mercurials, as the analysis of these materials is essentially a laboratory determination.

It is realized that any general recommendations cannot be accurate, but if the dip is not used for more than three or four batches of corms the error would not be of great practical importance. A calculation based on the graph of the results was made, and it was found that ½ oz. of Hortosan D.P. should be added after dipping each batch of 500 corms in 10 gallons of a solution of 2 oz. of Hortosan D.P. per 10 gallons for fifteen minutes.

Exposure of Corms to Volatile Fungicides.

The first use of a volatile material against a fungal disease was in the control of Blue Mould (*Peronospora tabaci*) of tobacco (Angell *et al* (1)). Subsequently, other related compounds have been used (59).

Various volatile materials were tested against the *Botrytis* of gladiolus. In preliminary tests the materials were tested against the fungus itself, using a slight modification of the method described by Oserkowsky (42). The effect of promising materials on healthy corms was then determined.

Methods.—Potato dextrose agar was poured into sterile 4-in. petri dishes. Before the medium had set a sterile 1-in. petri dish lid was placed in the centre of the medium. The plates were then inoculated with *Botrytis* and incubated for five days. One ml. of the volatile material was then pipetted into the small petri dish. The cultures were examined after 24, 48, and 72 hours and a small portion of the mycelium transferred to a potato dextrose agar slope. The slopes were then incubated and examined for growth.

Results.—The results are shown in Table 14:—

Table 14.

Chemical.	Results.		
	24 Hours.	48 Hours.	72 Hours.
Benzol	++	++	++
Toluene	++	++	++
Dioxan	−−	−−	+−
Paradichlorbenzene	−−	−−	+−

++ Colony flattened. No growth from subculture.
+− Colony flattened. Growth from subculture.
−− Colony unaffected. Growth from subculture.

Benzol and toluene were therefore effective in killing the fungus after exposure for 24 hours. Dioxan and paradichlorbenzene did not have any visible effect after exposure for 48 hours. After exposure for 72 hours both materials caused flattening of the colonies, but did not cause death of the fungus.

To determine the effect of exposure of gladiolus corms to benzol vapour, six corms of the variety, Mrs. S. A. Errey, were enclosed in a closed tin, with a layer of benzol at the bottom, for 24 hours. The corms were then planted.

The plants produced showed distortion, and the flowers which developed were severely distorted. On digging the plants, it was found that the corms produced were abnormal in shape (Plate V., fig. 10).

This method of treatment did not show promise of success, and experiments of this type were not continued.

Time of Digging.

Species of *Botrytis* have been frequently shown to be favored by cool humid conditions (2, 25). It was, therefore, anticipated that if corms were dug before the weather became cool and humid they would possibly escape the disease.

Miss Hawker found that narcissus bulbs showed heavier losses due to *Botrytis narcissicola* at low or moderate, rather than higher temperatures.

Methods.—Corms of the variety Picardy were planted in rows containing 100 corms. Four rows were planted at each of three periods separated by a month. They were dug six weeks after flowering.

The corms were then placed on wire-netting stretchers. After storage for six weeks the scales were removed from the corms, and the number of infected corms determined.

Results.—The corms were dug on the 2nd April, 23rd April, and on the 7th May. The number of infected corms is shown in Table 15:—

Table 15.

	Time of Digging.		
	Early.	Midseason.	Late.
Percentage	0·5%	27·1%	21·7%
Angle	4·06°	31·35°	27·72°

Whole experiment highly significant by F test.

Difference for significance at 1 per cent. level .. $6·77^{a}$

Difference for significance at 5 per cent. level .. $5·15^{a}$

Therefore by digging the corms early the disease was almost entirely avoided. Unfortunately only rainfall data are available for the Kalorama district and no data on humidity or temperature was obtained. A study of rainfall data for Kalorama in 1941 showed that some rain fell on each of the four days after digging the early plants, but then no further rain fell for ten days. Apparently these good drying conditions were unfavorable for the disease.

A study of rainfall data for Kalorama in the succeeding four seasons showed that if corms are dug before the second week of March, the rainfall is unlikely to be heavy following digging and therefore there is little chance of infection. All corms dug later should be dipped.

These observations were confined to the Kalorama district and no general recommendations are possible.

Varietal Resistance.

Answers to inquiries made among growers suggested that certain varieties were resistant to the disease. Therefore an experiment was conducted in the 1940-41 season to determine the relative susceptibility of various varieties to the disease.

Methods.—The corms were planted in rows, each containing 100 corms. There were four replicates of each variety and the plot was laid down as a randomized block. The corms were dug six weeks after flowering and placed on wire-netting stretchers in the same relative position as the plots in the field. After storage for six weeks the scales were removed and the percentage of infected corms determined.

Results.—The results are shown in Table 16:—

Table 16.

	Variety Used.						
	Golden Goddess	Pelegrina	Wolfgang v. Goethe	Miss New Zealand	Gate of Heaven	Picardy	Red Lory
Percentage ..	17·0%	1·5%	15·7%	0%	18·9%	27%	4·4%
Angle ..	25·04°	7·01°	23·35°	0°	25·79°	31·33°	12·18°

Whole experiment highly significant by F test.

Difference for significance at 1 per cent. level .. 3·53°

Difference for significance at 5 per cent. level .. 2·58°

Pelegrina, Miss New Zealand, and Red Lory all showed resistance to the disease, but Miss New Zealand was significantly more resistant than the other varieties, and Pelegrina was significantly more resistant than Red Lory.

It is interesting to note that the foliage of Pelegrina was severely infected with the fungus and had almost completely died down before digging. On the other hand Picardy showed some leaf spotting, but less than any other variety tested.

According to growers' reports, King Lear, Elinora, Mrs. S. A. Errey, Black Opal, Champlain, Don Bradman, and Rose Dawn are also resistant to the disease.

Discussion.

Botrytis corm rot is a disease which is favored by cool, humid conditions. Growers can therefore avoid infection of the corms by planting early, so that the corms are dug in the early autumn. However, as most growers desire to produce flowers for the cut-flower trade, in addition to corms, they prefer to plant portion of their stock at intervals throughout the season, so that all their flowers are not produced at the one time. Therefore they must have recourse to the other control measures described. Apart from the hope that in the future a large number of resistant varieties may be produced, the knowledge that certain popular varieties at present grown are resistant, will enable the grower to avoid the expense of unnecessarily dipping these varieties.

The results obtained with various dips applied at dipping time, show that very good control may be obtained with Hortosan D.P. Slightly less effective control can be obtained with corrosive sublimate, plus Agral II., and with Aretan. Zetan also gives good control. The higher degree of control obtained with Hortosan D.P. in the 1942-43 season than in the 1943-44 season may be due to the fact that the corms were dipped in muslin bags in the earlier season, and in hessian bags in the latter. Growers should preferably use muslin bags. It must be stressed that the corms be well washed before dipping, as many organic mercurials are inactivated by soil. Dipping must be carried out as soon as possible after digging. The suggestion for replenishing the Hortosan dip is tentative, and growers should not use the replenished dip for more than three or four batches of corms.

Observations suggest that the main method of overwintering of the disease is on infected corms. These usually bear abundant sclerotes and, as has been shown, these germinate under certain conditions to produce conidia. The conidia produced from this source would then infect flowers left in the field, and, as abundant conidia are rapidly produced on flowers, a heavy spore lead would be rapidly built up. Growers should therefore destroy infected corms by deep burial or by burning. They should not leave unwanted flowers in the field, but should cut them, and destroy them by burning or burial.

Good storage conditions are important and the maximum of ventilation should be provided. This is best achieved by using wire-netting stretchers arranged on racks. However, it is felt that good storage conditions, and attention to sanitation, as recommended in the preceding paragraph, will not eliminate the necessity for dipping, but rather all these measures are complimentary.

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Explanation of Plates.

Plate V.

Fig. 1.—Slightly infected corm.

Fig. 2.—Mummified corm. Note the sclerotes.

Fig. 3.—Infected corm showing mycelium and sclerotes of Botrytis.

Figs. 4-7.—Sections of corms showing progressive stages of infection.

Fig. 8.—Section of a corm showing infection commencing at the top of the core.

Fig. 9.—Conidiophore of Botrytis × 100.

Fig. 10.—Corms showing the effect of benzol on the growth of the corm in the subsequent crop.

Fig. 11.—The development of conidia from a sclerote × 10.

Fig. 12.—Artificial infection of a corm with Botrytis.

Plate VI.

Fig. 13.—Leaf infection with Botrytis.

Fig. 14.—Leaf from plant of same maturity as Fig. 13, but the plant was sprayed with Bordeaux mixture at flowering time.

Fig. 15.—Collar rot caused by Botrytis. Note the sclerotes on the collar region of the plant.

Figs. 16-18.—Progressive stages of infection of flowers with Botrytis. Note the conidia on Fig. 18.

Figs. 19-22.—The effect of light on Botrytis. The tubes illustrated in 19 and 20 were held at 23°C., but 19 was exposed to light and 20 kept away from light. Those illustrated in 21 and 22 were held at room temperature, and 21 exposed to light and 22 kept away from light.

Plate VII.

Fig. 23.—Section showing margin between healthy and diseased tissue. Note the accumulation of starch in infected tissue and the sharp line of demarkation between the healthy and diseased tissue. Stained with iodine and erythrosin. × 35.

Fig. 24.—Section showing an intermediate layer of low starch content between the healthy and diseased tissue. Note the accumulation of starch in the diseased tissue (lower right). Stained with iodine and light green. × 35.

Fig. 25.—Freehand section through an arrested lesion. Note the suberinised layer between the starch free layer (lower) and the normal tissue. Stained with iodine and Sudan III. × 42.

Fig. 26A.—Longitudinal section through an infected vascular bundle × 35.

Fig. 26B.—Enlargement of portion of 26A, showing apparently unaffected Xylem tissue and almost completely disorganized phloem tissue. Stained with Haidenhain's Haematoxylin and light green. × 85.

Fig. 27.—Transverse section through an infected vascular bundle. Note the disorganization of infected tissue and the sharp line of demarkation between healthy and diseased tissue. Stained with Haidenhain's Haematoxylin and light green. × 35.

Fig. 28.—Tangential section through an infected vascular bundle. Note the heavy fungal infection of the vascular bundle, and the absence of hyphae in the adjacent parenchymatous tissue. Stained with Haidenhain's Haematoxylin and light green. × 35.


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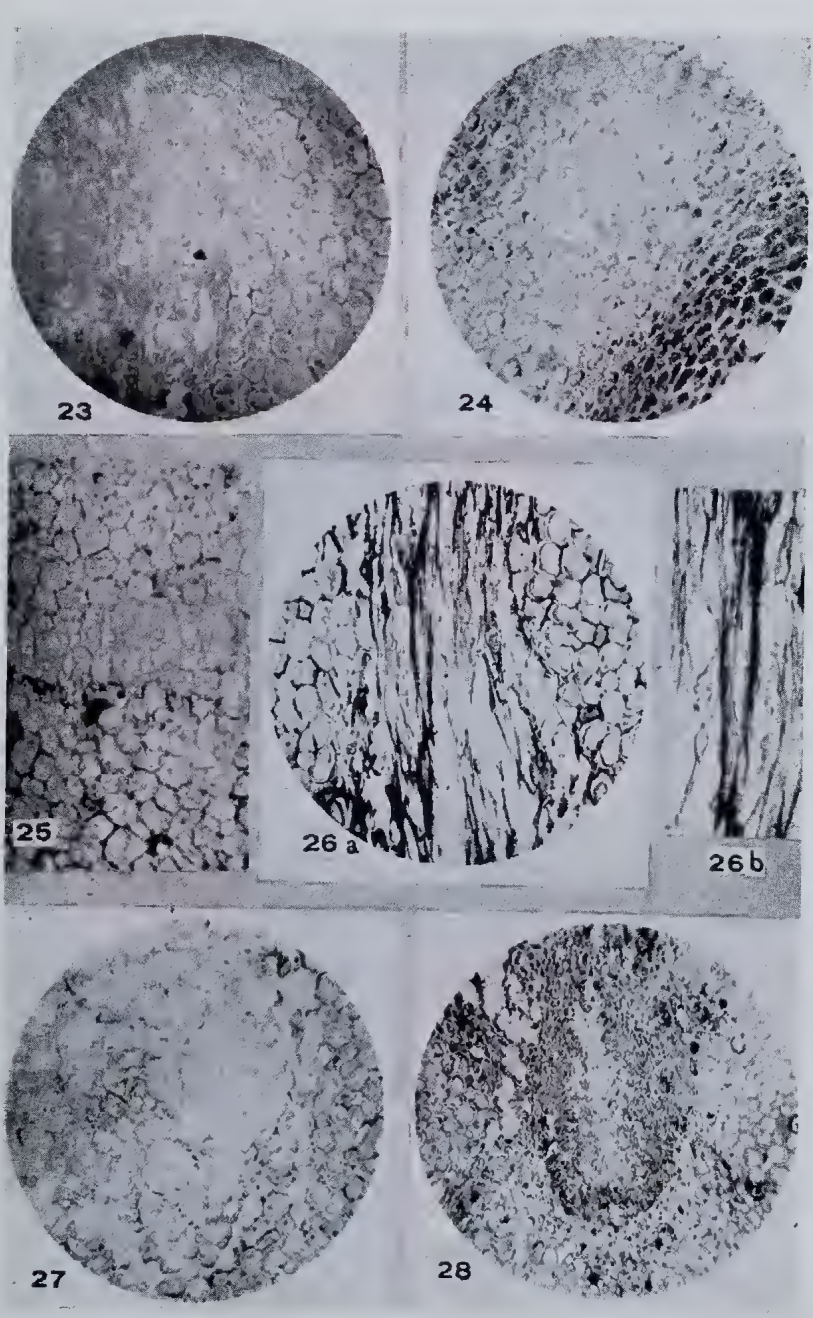
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